

A cross-sectional study of the association between cognitive impairment and haemoglobin levels in HIV-infected South Africans established on antiretroviral therapy.

Dissertation submitted in part fulfillment of the degree MMed (Psychiatry)

Candidate: Dr. John-Randel Vermaak

UCT student: VRMJOH005

Department of Psychiatry and Mental Health

University of Cape Town

Cell: 0824482191

E-mail: jrvermaak@gmail.com

Supervisor: Prof John Joska

Co-supervisor: Dr. Eric Decloedt

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DECLARATION

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ABSTRACT

Background

Sub-Saharan Africa, the epicenter of the global population of people living with HIV (PLHIV), is estimated to have more than 25 million PLHIV. In the era before the widespread availability of antiretroviral therapy (ART), anaemia (low serum haemoglobin) was a common clinical finding that was seen as a potential risk factor for HIV-associated neurocognitive impairment. The association between haemoglobin levels and neurocognitive function has not been assessed in a Sub-Saharan study population in the era of ART.

Methods

A cross-sectional secondary data analysis was performed to assess the association between serum haemoglobin level and neurocognitive function in 129 participants who had both neurocognitive test (global deficit score) and full blood count results performed as part of a randomised placebo controlled trial that evaluated the efficacy of lithium carbonate for the treatment of HIV associated neurocognitive disorders.

Results

The majority of our participants were female (87%) with a mean age of 37 ± 7.78 years. Participants were all established on ART with a median CD4 count of 495 cells/ μ L (IQR=315-629). The median haemoglobin level was 12.2 (IQR=11.6-13.00) and anaemia was present in 8.5%. Serum haemoglobin level was not associated with global deficit scores (GDS) and fewer years of education was the only independent risk association for GDS-defined neurocognitive impairment.

Conclusion

We found that in South Africans, who are established on ART, anaemia is less common than in the pre-ART era and importantly, that low-normal serum Hb levels do not present a risk for GDS-defined neurocognitive impairment. These findings are relevant as they show that aggressive management of low-normal Hb levels is not necessary provided individuals are otherwise clinically well and virally suppressed.

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ABBREVIATIONS

AIDS	–	Acquired Immunodeficiency Syndrome
ANI	–	Asymptomatic Neurocognitive Impairment
ART	–	Antiretroviral Therapy
CD4	–	Cluster of Differentiation 4
CI	–	Confidence interval
GDS	–	Global Deficit Score
GSH	–	Groote Schuur Hospital
HAD	–	Human Immunodeficiency Virus Associated Dementia
HAND	–	HIV Associated Neurocognitive Disorder
Hb	–	Haemoglobin
HIV	–	Human Immunodeficiency Virus
HIV-1	–	Human Immunodeficiency Virus Type 1
IHDS	–	International HIV Dementia Scale
IQR	–	Inter Quartile Range
MCH	–	Mean Corpuscular Haemoglobin
MCV	–	Mean Corpuscular Volume
NHLS	–	National Health Laboratory Service
OR	–	Odds Ratio
PLHIV	–	People Living with Human Immunodeficiency Virus
SD	–	Standard Deviation
UCT	–	University of Cape Town
VL	–	Viral load

CHAPTER 1

1 Background

1.1 Human immune deficiency virus (HIV) associated neurocognitive disorders - an introduction

The global estimate of people living with HIV (PLHIV) is 36.9 million (UNAIDS 2015 Factsheet). Sub-Saharan Africa continues to carry the largest burden of this disease with an estimated 25.8 million PLHIV. In the last two decades major strides have been made with regards to improved access and the early initiation of antiretroviral therapy (ART). This has had an impact on the epidemiology and presentation of clinical syndromes associated with HIV. Previously, typical complications of HIV related to severe immune compromise. However, in the modern era of ART, HIV has increasingly been associated with conditions related to chronic low-grade immune dysregulation, accelerated cellular aging and the negative effects of long term ART use. (1)

This change in the clinical picture of HIV is particularly evident with regards to the neurocognitive disorders associated with HIV. In the 1980's and early 1990's advanced HIV infection was frequently complicated by a progressive subcortical dementia with psychomotor slowing and severe cognitive impairment, a condition then called HIV-dementia (HAD). (2) More recently, since the advent of ART, the incidence of HIV-dementia has been reduced. (3) However, as ART use became more widespread, it was evident that milder forms of neurocognitive impairment were common amongst PLHIV. It was suggested that an estimated 50% of PLHIV who are clinically stable and receiving ART could be expected to have neurocognitive impairment on objective neuropsychological testing. (4) The emergence of this spectrum of neurocognitive impairment saw an expansion of the nosology used to describe HIV associated neurocognitive impairment. (5)

HIV associated neurocognitive disorders (HAND) now serves as an umbrella term which can be further defined on the basis of both the degree of functional impairment and the associated objective neuropsychological test findings. As the term suggests, the individual with asymptomatic neurocognitive impairment (ANI) does not subjectively experience any impairment in everyday functioning but neuropsychological testing demonstrates a performance of at least 1.0 standard deviation (SD) below the mean for age-education-appropriate norms. In mild neurocognitive disorder (MND) the individual has some degree of interference with day-to-day cognitive functioning and neuropsychological testing demonstrates a performance of at least 1.0 SD below the mean for age-education-appropriate norms. HIV- associated dementia (HAD) represents the most severe form of impairment and the patient typically has marked interference

with daily functioning with neuropsychological testing demonstrating a performance of greater than 2.0 SD below demographically corrected means. (5)

Epidemiological data suggests that amongst PLHIV who are receiving ART approximately: 30% have ANI, 20% have MND and 2% - 8% have HAD. (6) The overall figure of HAND is expected to rise even further as the global HIV positive population ages. (6)

HAND is associated with significant morbidity and has been shown to have a negative impact on everyday function, employability as well as ART adherence. (7, 8, 9) There is no definitive adjuvant treatment for the management of HAND in PLHIV who are clinically stable on ART. (6, 10)

1.2 The pathophysiology of HAND

HIV enters the brain in the early phase of systemic infection. (11) The most widely accepted hypothesis of neuro-invasion suggests that HIV crosses the blood brain barrier through infected macrophages and microglia. (6) Since neurons do not express the glycoproteins that render cells susceptible to HIV infection, HIV cannot directly infect neurons. The neurodegenerative mechanisms, which underlie HAND, must therefore occur via an alternative mechanism. Two non-mutually exclusive models of neuronal injury have been suggested: the direct injury hypothesis and the bystander effect hypotheses.

The direct injury hypothesis proposes that viral proteins such as glycoprotein 120 (gp120) are able to interact with chemokine and N-Methyl-D-Aspartate (NMDA) receptors. This activates intracellular secondary messenger systems which may initiate apoptotic pathways and give rise to neuronal injury. Gp120 can also stimulate non-neuronal cells to produce tumor-necrosis factor (TNF). TNF activates further cellular cascades that ultimately lead to neuronal cell death. (6)

Two additional viral proteins, Tat and Vpr, have also been suggested as potential contributors to neuronal injury. In a similar fashion to TNF, Tat and Vpr can disrupt intracellular processes and thereby induce intracellular injury. (6)

The bystander effect hypothesis suggests that HIV infection in the central nervous system activates inflammatory pathways that ultimately lead to neuronal injury. HIV activates microglia and brain macrophages. These activated immune cells then initiate an inflammatory cascade through the release of TNF and other pro-inflammatory cytokines. This inflammatory response can in turn activate uninfected cells and in doing so, lead to the amplification of HIV-induced neurotoxicity. In addition, it is thought that this

inflammatory response can impair the function of astrocytes that usually serve as neuronal support cells. (6)

The widest held aetiological understanding of the HAND disorders views the degrees of clinical severity as occurring along a spectrum that correlates with the underlying degree of neurodegenerative insult. (6) It remains uncertain why PLHIV are more likely to experience HAND but current evidence suggests a complex, multifactorial risk profile which includes both viral and host-related risk factors.

1.3 Risk factors associated with HAND

Viral related factors

1.3.1 Nadir CD4

One of the most consistent risk factors associated with HAND is the severity of immune suppression as measured by the lowest (nadir) cluster of differentiation 4 T lymphocyte (CD4) count during HIV infection. (12, 13) A period of severe immunosuppression prior to ART initiation appears to present an enduring risk for neurocognitive impairment even when ART has led to immune reconstitution. (13)

It has been argued that severe immunosuppression invariably leaves the affected individual with neuronal injury. (14) Even though this initial neuronal damage may not be sufficient to induce functional impairment, it increases the individual's susceptibility for developing symptomatic neurocognitive impairments. (14)

It should be noted that current ART guidelines advocate for the early initiation of ART. As such, severe immunosuppression has become less common amongst PLHIV. Due to this change, the strength of association between nadir CD4 and HAND has been reduced, highlighting the importance of additional HAND risk factors in the ART era. (6)

Host related factors

1.3.1 Genetic factors

Apolipoprotein E is a protein that is involved in lipid metabolism in the brain. Individuals who are homozygous for the E4 allele of apolipoprotein E (Apo E4) have an increased risk of developing Alzheimer's disease. (15) Apo E4 status has also shown some association with HAND in selected HIV-infected cohorts, particularly amongst older PLHIV. (16) This association has not been clearly replicated amongst younger PLHIV. (17, 18)

The first genome wide association study for HAND conducted in a North American, HIV-infected, male population did not find any association between genetic susceptibility loci and HAND. (19) Therefore, although it is possible that genetic predisposition may

play a role in the etiology of HAND, current evidence has not identified clear genetic risk factors. (18)

1.3.2 Older age

In HIV-infected populations, older age has been associated with an increased risk of HAND. (20-22) Furthermore, as access to ART improves, HIV-infected populations grow older and therefore, the incidence of HAND increases in these populations. (4)

It is well known that older age is a risk factor for age-related neurodegenerative conditions such as Alzheimer's disease and vascular dementia. Some researchers have suggested that the risk association and mechanism underlying cognitive impairment and age is similar in HIV-infected and HIV-uninfected populations. (23) Contrastingly, others have argued that HIV infection and/or ART accelerates the physiological aging process and as a result, neurodegenerative conditions may present earlier in PLHIV. (24)

An additional factor that may be contributing to the cognitive decline noted in older individuals living with HIV is the increased frequency of comorbid vascular and metabolic conditions that have a well-established association with older age.

1.3.4 Metabolic abnormalities and vascular disease

The metabolic syndrome is a cluster of metabolic abnormalities that include hyperglycemia attributable to insulin resistance, hypertension, obesity, and dyslipidemias (elevated triglycerides and low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol). These metabolic abnormalities are common complications in individuals receiving ART. (25, 26) Metabolic abnormalities have been associated with neurocognitive impairment in HIV-uninfected populations. (27) For PLHIV, diabetes mellitus and increased central obesity appear to be of particular importance with regards to HAND risk. (28)

1.3.5 Anaemia as potential risk factor for HAND

Anaemia is the most common hematological abnormality in PLHIV with previously reported prevalence rates as high as 90% in the pre-ART era. (29) The literature on anaemia reflects that different haemoglobin (Hb) levels are used as cutoff value to define anaemia. Although anaemia can be defined as serum Hb value below the accepted population norms (<11.5g/dL in females and <13g/dL in males), most international and South African studies have used a more robust clinically significant cutoff value of <10g/dL, regardless of sex.

As with HAND, the introduction of ART markedly reduced the incidence and prevalence of anaemia in PLHIV. In one of the first studies that assessed anaemia in a population receiving ART, the prevalence of anaemia was 11%. (30) Others have since reported prevalence rates of 5.4% and 7.2% amongst North American women living with HIV. (19, 31)

Prevalence rates for anaemia amongst South Africans receiving ART are limited. In a South African study that screened more than 10000 individuals prior to ART initiation, the overall prevalence of anaemia (Hb <10g/dL) was 25.8%. (32) This same study group later reported that in more than 80% of cases, pre-ART, anemia improved on ART. (33) Together these results suggest that in South Africa, the prevalence of anaemia amongst PLHIV, who are on established ART, is in the region of 5% - 10%.

The pathophysiology of anaemia in PLHIV is thought to be multifactorial. The most common form of anaemia in HIV is a normochromic normocytic anaemia with a low reticulocyte count, normal iron stores and impaired erythropoietin response. This pattern of anaemia is typical of anaemia of inflammation. It has been proposed that this form of anaemia is similar to that seen in anaemia of the elderly. (34) It is suggested that it is accelerated senescence and immune dysregulation associated with HIV that are the major pathophysiological mechanisms underlying the majority of HIV associated anaemia. (35)

Actively replicating HIV appears to suppress the body's normal physiologic response to anaemia. (36) In addition auto antibodies against erythropoietin has been demonstrated in HIV. (37)

Nutritional deficiencies, also more common in PLHIV, often result in anaemia. Namely, iron deficiency, associated with microcytic anaemia, and vitamin B12/folate deficiency is typically associated with macrocytic anaemia. Opportunistic infections and malignancies associated with HIV can also cause anaemia. (35) Lastly drugs used in HIV can in themselves result in anaemia including antiretroviral drugs, prophylactics for opportunistic infections and the drugs used in the treatment of opportunistic infections. (35)

To explore the literature on a possible association between anaemia and HAND, a PubMed search was conducted using the following search terms: HIV, human immunodeficiency virus, acquired immune deficiency syndrome, AIDS (AND) neurocognitive impairment, neurocognitive disorder, HAND (AND) haemoglobin, hemoglobin, anemia and anaemia. This search yielded twenty-five results. Screening of the titles showed four of these articles to be relevant. (38-41). Further scrutiny of articles that had been cited by these four studies revealed one additional relevant study. (42) Since negative findings are rarely reported in the title of published articles, it was difficult to identify studies where anaemia was not associated with HAND. By screening

the major published literature on the risk factors for HAND in the era of ART, one study was identified where no association between Hb and HAND was found. (43) A summary of the findings of these six studies is shown in Table 1.

Four North American studies and one study from Cameroon have shown an association between anaemia and HAND. (38-41) In the most recent of these, Kallianpur et al. of the CHARTER study group reported that in a North American cohort, where 70% of the study population was receiving ART, mean corpuscular volume and mean corpuscular haemoglobin were positively associated with all forms of HAND. Additionally, anaemia was an independent predictor for the later development of HAND (Global Deficit Score \geq 0.5 or meeting Frascati criteria) during a 72-month follow-up period. (41)

Table 1. Studies of the association between low haemoglobin and HAND

Author, year published	Country	Study size	Participants	Study Design	Clinical characteristics of study sample	Definition and Prevalence of Anaemia	Findings
J McArthur et al. 1993	North America	492	Male; Mean age 37yrs	Longitudinal cohort	All had AIDS defining illness suggesting severe immune compromise	Not reported, haemoglobin recorded as continuous variable	On multivariate analysis an increase in haemoglobin (per additional 2g/dl) 1- 6 months prior to ART showed relative hazard (95% CI) of 0.6 (0.38-0.96) for HAD* on follow up
A Qureshi et al. 1998	North America	19462	Predominantly male (85%); 95% younger than 50yrs	Longitudinal folder review	45% CD4 <200cells/μL; 45% received zidovudine	Anaemia defined as: women Hb <10g/dL & men Hb <11g/dL Prevalence 27%	Low haemoglobin showed relative risk (95%CI) of 2.8 (2.4-3.2) for HAD**
Tozzi et al. 2005	Italy	432	Predominantly male (71%); Mean age 39yrs	Survey	Mean CD4 count 301 cells/μL; 76% receiving ART	Not reported, haemoglobin recorded as continuous variable	Haemoglobin showed no association with neurocognitive impairment as measured by a neuropsychological test battery

Njamnshi et al. 2009	Cameroon	185	Predominantly female (67%); Mean age 37yrs	Cross-sectional analysis	Mean CD4 count 265 cells/ μ L;	Anaemia defined as: Hb <10g/dL Prevalence 35%	Anaemia showed odds ratio(95% CI) of 1.16 (1.00-1.31) for IHDS*** \leq 10
M Marquine et al. 2014	North America	601	Predominantly male (88%); Mean age 42yrs64	Longitudinal cohort	64% had AIDS defining illness; 63% received ART	Anaemia defined as Hb <10g/dL Prevalence 2%	Anaemia showed adjusted odds ratio (95%CI) of 2.24(1.01-5.00) for global neurocognitive impairment****.
A Kallianpur et al. 2015	North America	1261	Predominantly male (77%); Mean age 43yrs	Longitudinal cohort	Median CD4 nadir 181 cells/ μ L; 70% receiving ART	Anaemia defined as: women Hb <11.5g/dL & men Hb <13 g/dL Prevalence 15%	Baseline anaemia showed adjusted hazards ratio (95%CI) of 1.55(1.13–2.12) for HAND***** *during 72-month follow up

*Clinical diagnosis, see McArthur et al. 1993, ** Centre for Disease Control's 1987 clinical criteria for HAD, *** International HIV Dementia Scale, **** Neurocognitive impairment defined as: Global Deficit Score \geq 0.5, ***** HAND defined as: Global Deficit Score \geq 0.5 or meeting Frascati criteria

It should be noted that an exact causal mechanism through which anaemia contributes to development of neurocognitive impairment has not been demonstrated however, the following mechanisms have been suggested. (44)

Haemoglobin is an essential determinant of the oxygen delivery capacity of blood. Therefore, although not clinically proven, it is possible that anaemia can be viewed as a state of chronic cerebral hypoxia which may cause, or worsen, neurocognitive impairment. (45) It has been shown that acute onset hypoxia causes cognitive impairment and that supplementary oxygen administration can improve cognition. (46, 47) In addition, other chronic conditions associated with suboptimal cerebral oxygen delivery such as obstructive sleep apnoea and chronic hypotension have a negative impact on neurocognitive performance. (48, 49) Together, findings provide circumstantial support to the general hypothesis that anaemia, on the basis of low grade chronic hypoxia, can lead to neurocognitive impairment.

Some of the specific aetiological mechanisms that give rise to anaemia can theoretically be linked with impaired cognition. Examples include: a) reduced erythropoietin, b) iron deficiency, c) vitamin B12/folate deficiency, d) systemic inflammation associated with chronic disease and e) accelerated senescence associated with HIV.

- a) Erythropoietin is known for its function in regulating red blood cell production. Additionally, a growing body of evidence shows that erythropoietin is neuro-protective. (50) Erythropoietin protects the CNS against oxidative stress, can possibly reduce tau phosphorylation and protects against- β -amyloid-induced apoptosis. (51-53) Anaemia is associated with low erythropoietin and therefore it is plausible that in the presence of anaemia erythropoietin related neuro-protection is reduced. (35)
- b) The presence of iron deficiency has been associated with impaired neurocognitive performance. (54) As noted, haemoglobin is a cellular protein that regulates the oxygen carrying capacity of erythrocytes. Iron is essential in the production of haemoglobin and therefore it is possible, in the context of low iron levels, that the blood's oxygen carrying capacity will be reduced. This may in turn lead to a degree of brain hypoxia. Additionally, iron is an important micronutrient for normal cellular function and thus low iron levels can also be directly responsible for impaired neuronal function. (55)
- c) Vitamin B12 is another essential micronutrient important for erythrocyte and cognitive function. Vitamin B12 deficiency is known to be associated with both Alzheimer's dementia and vascular dementia. (56) Characteristically, B12 deficiency is associated with the classical hematological feature of macrocytic anaemia. As in the case of erythropoietin and iron deficiency, B12-related anaemia can be thought of

as both a causal factor as well as a proxy marker of an additional neuropathological process.

- d) The presence of anaemia in itself can also be considered as a marker of general ill-health. This is particularly relevant in the setting of HIV since HIV associated anaemia is strongly associated with more advanced immune compromise. Anaemia may therefore serve as a proxy marker of immunosuppression in PLHIV, a well-known risk association of HAND.
- e) Lastly, anaemia may be a marker of the accelerated senescence associated with HIV. Since this accelerated cellular aging process has also been suggested to be a risk factor for HAND, it is possible that anaemia and HAND can co-occur on the basis of a shared aetiological mechanism.

The association between Hb/anaemia and HAND has not been assessed in a South African study population established on ART. It is important to highlight some of the differences that exist between HIV infected populations in Sub-Saharan Africa and the study cohorts shown in Table 1.

Firstly, certain demographic characteristics are unique to African cohorts. The most notable of these is that, in Africa, a large proportion of the total HIV infected population is female. In contrast, most study populations in North America consist of Caucasian males. Clear differences exist between the normal Hb distributions for females as compared to males and these differences may impact on the relationship between Hb level and physiological performance.

There are also socio-economic differences between African and North American populations. The majority of the PLHIV in Sub-Saharan Africa face severe socioeconomic stressors and may be at higher risk of micronutrient deficiencies that can give rise to anaemia. In addition, co-infection with opportunistic infections such as pulmonary tuberculosis and helminth infections, which can both cause anaemia, is common in PLHIV in Sub-Saharan Africa.

Finally, differences exist with regards to the predominant clade of HIV-1. In sub-Saharan Africa the clade C subtype is responsible for the vast majority of HIV infections whereas in North America clade B accounts for most cases of infection. It is possible that these two clades of HIV may have different virulence with regards to their haematological effects.

To the best of our knowledge, it is currently uncertain whether anaemia harbors any clinically significant risk for neurocognitive impairment in South Africa. We consider this as an important research question because if anaemia is indeed a risk factor for

HAND, then it will fall into the rare category of potentially modifiable risk factors. This would have clear implications for clinical practice in South Africa.

Our aim with this present study was to investigate the potential association between anaemia and HAND in a HIV infected South African study population where participants were clinically stable and on established ART.

2 Study hypothesis, aim and objectives

2.1 Study Hypothesis

Haemoglobin levels are associated with neurocognitive impairment in PLHIV in South Africa.

2.2 Aim

To determine the association between haemoglobin level and neurocognitive impairment in PLHIV who are virally suppressed and have received at least 6 months of ART.

2.3 Objective

To perform a cross-sectional analysis of an existing dataset (57) to explore whether haemoglobin, mean corpuscular volume (MCV) or mean corpuscular haemoglobin (MCH) is associated with neurocognitive function, as assessed by global deficit score (GDS).

3 Methodology

3.1 Study Design

We conducted a cross-sectional secondary data analysis of a randomised placebo controlled trial that evaluated the efficacy of lithium carbonate for the treatment of HAND. (57)

3.2 Study Population

The study population consisted of PLHIV who were established on ART for at least 6 months. Study population details have been published. (57)

3.3 Sample size

One-hundred and forty-seven individuals were screened. A secondary data analysis was done of the 129 individuals who had both neurocognitive screening and phlebotomy for FBC performed. (Figure 1) An estimate of statistical power based on sample size suggested that our study was powered at 80% certainty.

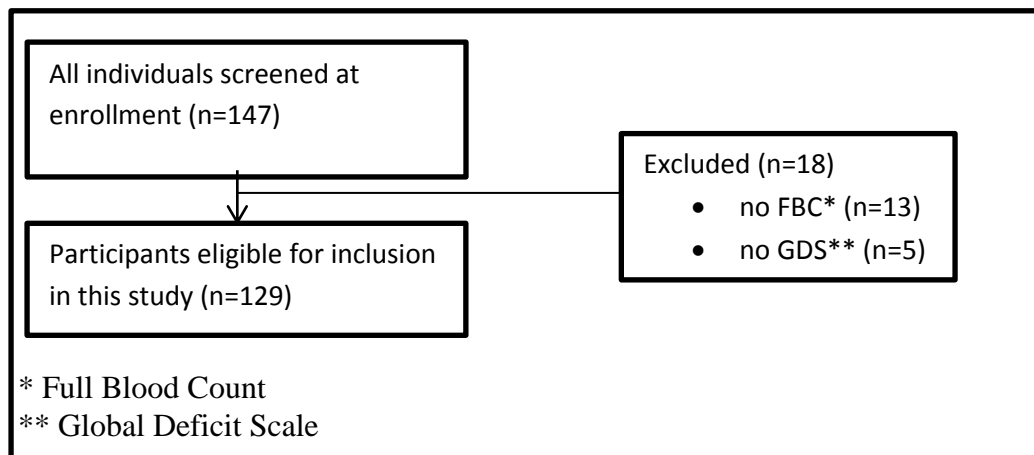


Figure 1: Summary showing how participants were included in this study

3.4.1 Inclusion criteria

Study participants had to have had neurocognitive screening and full blood counts done. For consent purposes, they had to be older than 18 years and cognitively well enough to provide informed consent. To minimize confounders, they had to be otherwise clinically well with systemic viral suppression (HIV PCR <400 copies/mL).

3.5 Exclusion criteria

Exclusion criteria have been published. (57) Of note, participants were excluded if neuropsychiatric disorders, serious psychiatric symptoms or imaging structural abnormalities were present.

3.6 Participant recruitment

Participants were actively recruited in the waiting rooms of two HIV treatment clinics in Cape Town. (57)

3.7 Withdrawal of participants

Participants were free to withdraw from the study at any stage. (57)

3.8 Participants' obligations

Participants had to notify the investigator if they were unable to follow any study procedures, experienced any adverse events, ingested any new drugs or received instructions from a health care provider to change any chronic medication dosages. (57)

3.9 Study procedure

As published. (57)

For the purpose of this study it was important to note that the following blood investigations were done:

- Full blood count including differential count and red cell indices
- Chemistry: thyroid stimulating hormone (TSH) (T3 and T4 was performed if TSH was abnormal), sodium, potassium, calcium, urea and creatinine
- Plasma viral load
- CD4+ count
- Rapid plasma reagin test (RPR)
- Vitamin B12

3.10 Haematological analyses and definition of anaemia

3.10.1 Haematological analyses

Phlebotomy for full blood counts was only done at study entry. All blood samples were collected in appropriate ethylenediaminetetraacetic acid (EDTA) tubes. An accredited South African pathology laboratory service provider was used for the processing of these samples. The automated processing of haematological samples was done by Advia machines. All results were signed out by qualified haematological pathologists.

3.10.2 Definition of anaemia

A stringent definition of anaemia, as reflected by a serum haemoglobin level of < 10 g/dL, was used. This definition was in keeping with the definition used by most South African studies investigating anaemia in HIV positive populations and was also in keeping with the WHO toxicity grading systems definition of anaemia. (33)

3.11 Determination of GDS

The GDS has been validated for use in HIV and summarises the neuropsychological test results of selected cognitive domains and adjusts for age, education, gender and ethnicity (Carey, 2004). Neurocognitive impairment was defined as a GDS score of greater than 0.5. In our statistical analysis we looked at associations with GDS as a continuous variable. In addition, we explored group differences between participants with normal or abnormal GDS scores.

3.12 Neuropsychological test battery

The neuropsychological test battery was performed by trained technicians. This battery was designed to be sensitive to the neurological functions affected by HIV. The standard battery includes the following:

- Attention: the Mental Alternation Test (MAT) and the Mental Control Test (MCT);
- Learning and memory: the Hopkins Verbal Learning Test (HVLT) and the Brief Visuospatial Memory Test (BVM);
- Motor speed: Finger Tapping (FT) and the Grooved Pegboard Test (GP);
- Psychomotor speed: Trail Making Test part A (TMTA), Colour Trails Test 1(CT1) and Digit Symbol-Coding (DSC);
- Executive function: Colour Trails Test 2 (CT2), Stroop Colour-Word test (SCW), Wisconsin Card-Sorting Test (WCST);
- Language: category fluency;
- Depression score: Center for Epidemiologic Studies Depression scale (CES-D)

3.13 Data management

The dataset used for this research did not contain the subject's personal identities. Steps involved in data management, including those relating to the development and management of the database were performed in accordance with standard operating procedures that were consistent with regulatory requirements. The data set for this secondary analysis was password protected. The data will be stored for 5 years following submission of this MMed.

3.14 Statistical Plan

In keeping with the aims of our study, the following analysis was conducted:

A descriptive analysis of the risk associations for HAND in participants who were screened for entry into the Li in HAND RCT study.

Univariate analysis was performed to assess the association between GDS and variables of interest including age, level of education, CD4 count, Red cell count, Hb, MCV, MCH, TSH and Vit B12. T-tests and Chi-square tests were used as applicable. An attempt was made to log transform all nonparametric data in order to use parametric statistical tools. Where such transformations were not possible nonparametric statistical tools were used- namely Mann-Whitney and Fisher Exact tests. Statistical significance was set at the .05 level.

All variables that reached significance on univariate analysis were included in a series of regression analyses, using GDS as the dependent variable.

In addition to this, the data was binarized on the basis of GDS using 0.5 as cutoff.

Descriptive and cross-sectional analysis was performed by J Vermaak using STATA/IC 11. A final data analysis was supervised by a UCT based statistician, Michelle Henry.

3.15 Ethical considerations

3.15.1 Informed consent

As published. (57)

3.15.2 General ethical principles

The protocol for this sub-study was presented and approved in a peer-reviewed manner. Ethical approval was obtained from the University of Cape Town's Human Research Ethics Committee, HREC 530/2017.

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CHAPTER 2

ARTICLE TITLE

A cross-sectional study of the association between cognitive impairment and haemoglobin levels in HIV-infected South Africans established on antiretroviral therapy.

AUTHORS

J Vermaak, MBChB, MSc (Med), FC Psych (SA)

E Decloedt, MBChB, BSc Hons (Pharmacology), MMed (Clinical Pharmacology), FCCP (SA), PhD

J Joska, MBChB, MMed (Psych), FC Psych (SA), PhD

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A cross-sectional study of the association between cognitive impairment and haemoglobin levels in HIV-infected South Africans established on antiretroviral therapy.

J Vermaak,¹ MBChB, MSc (Med), FC Psych (SA)

E Decloedt,² MBChB, BSc Hons (Pharmacology), FCCP (SA), MMed (Clinical Pharmacology), PhD

J Joska,¹ MBChB, MMed (Psych), FC Psych (SA), PhD

¹ Department of Psychiatry and Mental Health, University of Cape Town, South Africa

² Division of Clinical Pharmacology, University of Stellenbosch, South Africa

Corresponding author: J Vermaak (jrvermaak@gmail.com)

Background

Sub-Saharan Africa, the epicenter of the global population of people living with HIV (PLHIV), is estimated to have more than 25 million PLHIV. In the era before the widespread availability of antiretroviral therapy (ART), anaemia (low serum haemoglobin) was a common clinical finding that was seen as a potential risk factor for HIV-associated neurocognitive impairment. The association between haemoglobin levels and neurocognitive function has not been assessed in a Sub-Saharan study population in the era of ART.

Methods

A cross-sectional secondary data analysis was performed to assess the association between serum haemoglobin level and neurocognitive function. One hundred and twenty-nine participants who had both neurocognitive test (global deficit score) and full blood count results performed as part of a randomised placebo controlled trial were included.

Results

The majority of our participants were female (87%) with a mean age of 37 ± 7.78 years. Participants were all established on ART with a median CD4 count of 495 cells/ μ L (IQR=315-629). The median haemoglobin level was 12.2 (IQR=11.6-13.00) and anaemia was present in 8.5%. Serum haemoglobin level was not associated with global deficit scores (GDS) and fewer years of education was the only independent risk association for GDS-defined neurocognitive impairment.

Conclusion

We found that in South Africans, who are established on ART, anaemia is less common than in the pre-ART era and importantly, that low-normal serum Hb levels do not present a risk for GDS-defined neurocognitive impairment. These findings are relevant as they show that aggressive management of low-normal Hb levels is not necessary if individuals are otherwise clinically well and virally suppressed.

Introduction

Sub-Saharan Africa, the epicenter of the global population of PLHIV, is estimated to have more than 25 million PLHIV. In the last two decades major strides have been made with regards to improved access and the early initiation of ART. This has impacted on both the epidemiology and presentation of clinical syndromes associated with HIV. Pre-ART typical complications of HIV were related to severe immune compromise. However, in the modern era of ART, HIV is now more frequently associated with conditions related to chronic low-grade immune dysregulation, accelerated cellular aging and the negative effects of long term ART use. (1)

This change in the clinical picture of HIV is particularly evident with regards to the neurocognitive disorders associated with HIV. In the pre-ART era advanced HIV infection was often complicated by a progressive subcortical dementia with psychomotor slowing and severe cognitive impairment, a condition then called HIV-dementia (HAD). (2) With the advent of ART, the incidence of HIV-dementia has been reduced. However, it is now evident that milder forms of neurocognitive impairment are common amongst PLHIV. (3) In fact, it has been estimated that 50% of PLHIV, who are clinically stable and receiving ART, may be found to have neurocognitive impairment on objective neuropsychological testing. (4) The awareness of this spectrum of neurocognitive impairment in PLHIV has seen an expansion of the nosology used for the HIV associated neurocognitive disorders (HAND). (5) HAND now serves as an umbrella term which can be further defined on the basis of both the degree of functional impairment and the associated objective neuropsychological test findings. As the term suggests, the individual with asymptomatic neurocognitive impairment (ANI) does not subjectively experience any impairment in everyday functioning but neuropsychological testing demonstrates a performance of at least 1.0 standard deviation (SD) below the mean for age-education-appropriate norms. In mild neurocognitive disorder (MND) the individual has some degree of interference with day-to-day cognitive functioning and neuropsychological testing demonstrates a performance of at least 1.0 SD below the mean for age-education-appropriate norms. HIV-associated dementia (HAD) represents the severest form of impairment and the patient typically has marked interference with daily functioning with neuropsychological testing demonstrating a performance of greater than 2.0 SD below demographically corrected means. (5)

Epidemiological data shows that amongst PLHIV who are receiving ART approximately: 30% have ANI, 20% have MND and 2%-8% have HAD. (6) The overall figure of HAND is expected to rise even further as the global HIV positive population ages. (6) This is of serious concern because HAND is associated with significant morbidity and has been shown to have a negative impact on everyday function, employability and ART adherence. (7, 8, 9) Of further concern is the lack of a definitive adjuvant treatment for the management of HAND in PLHIV who are clinically stable on ART. (6, 10)

Anaemia and HAND

Anaemia is the most common hematological abnormality in PLHIV and in the pre-ART era prevalence rates as high as 90% were reported. (11) As with HAND the introduction of ART markedly reduced the incidence and prevalence of anaemia to between 5% and 10% in PLHIV who are established on ART. (12, 13, 14)

The aetiological mechanisms underlying anaemia in HIV are vast. The most common form of anaemia in HIV is a normochromic normocytic anaemia with a low reticulocyte count, normal iron stores and impaired erythropoietin response. This pattern of anaemia is typical of chronic inflammation and it is therefore thought that the immune dysregulation associated with HIV is the major pathophysiological mechanisms underlying most HIV associated anaemia. (15) Apart from the above, HIV may have a direct suppressive effect on the cytokine cascade responsible for normal erythropoiesis. Actively replicating HIV appears to suppress the body's normal physiologic response to anaemia. (16) Auto-antibodies against erythropoietin, the hormone that stimulates erythropoiesis, have also been demonstrated in HIV. (17) The common opportunistic infections and the malignancies that affect PLHIV can also contribute to anaemia. (15) Nutritional deficiencies, also more common in PLHIV, often result in anaemia. Namely, iron deficiency, associated with microcytic anaemia, and vitamin B12/folate deficiency which is typically associated with macrocytic anaemia. Lastly drugs used in HIV can in themselves result in anaemia including antiretroviral drugs, prophylactics for opportunistic infections and the drugs used in the treatment of opportunistic infections. (15)

In the pre-ART era, a risk association was shown between anaemia and HAND. (18-21) More recently, Kallianpur et al. of the CHARTER study group reported that in a North American cohort, where 70% of the study population was receiving ART, mean corpuscular volume and mean corpuscular haemoglobin were positively associated with all forms of HAND. Additionally, anaemia was an independent predictor for the later development of HAND (Global Deficit Score ≥ 0.5 or meeting Frascati criteria) during a 72-month follow-up period. (21)

The exact mechanism through which anaemia can contribute to the development of neurocognitive impairment has not been demonstrated but the following mechanisms have all been suggested

As the essential determinant of the oxygen delivery capacity of blood, it is possible that anaemia might represent a state of chronic low-grade cerebral hypoxia. (22, 23) This hypothesis has not been tested in vivo and it is uncertain at which Hb level the hemodynamic system's ability to optimally oxygenate the brain is compromised. However, it has been shown that acute onset hypoxia causes cognitive impairment and that supplementary oxygen administration can improve cognition. (24, 25) In addition, other chronic conditions associated with suboptimal cerebral oxygen delivery such as obstructive sleep apnoea and chronic hypotension have a negative impact on neurocognitive performance. (26, 27)

Apart from the anaemia itself, some of the aetiological mechanisms that give rise to anaemia can theoretically be linked with neurocognitive function. Examples include: systemic inflammation associated with chronic disease, reduced erythropoietin, iron deficiency, vitamin B12 deficiency, and accelerated senescence associated with HIV. HIV associated anaemia is strongly associated with more advanced immune compromise. To this extent anaemia may serve as a proxy marker of immunosuppression in PLHIV. Advanced immunosuppression is a well-known risk association of HAND.

Erythropoietin is known for its function in regulating red blood cell production. There is also a growing body of evidence showing that erythropoietin is neuro-protective. (28) Erythropoietin protects the CNS against oxidative stress, can possibly reduce tau phosphorylation and protects against- β -amyloid-induced apoptosis. (29, 30, 31) Anaemia is associated with low erythropoietin and therefore it is plausible that in the presence of anaemia erythropoietin related neuro-protection is reduced. (15)

Iron deficiency has also been associated with impaired neurocognitive performance. (32) Iron is an important micronutrient for normal cellular function and therefore low iron levels can also be directly responsible for impaired neuronal function. (33) Additionally, iron is also important for its role in the production of haemoglobin and therefore iron deficiency anaemia may be associated with HAND on the basis of anaemia being a proxy marker of systemic iron deficiency.

Vitamin B12 is another essential micronutrient important for erythrocyte and cognitive function. Vitamin B12 deficiency is known to be associated with both Alzheimer's dementia and vascular dementia. (34) Characteristically though, B12 deficiency is also associated with macrocytic anaemia. As in the case of erythropoietin and iron deficiency, B12-related anaemia can be thought of as both a causal factor as well as a proxy marker of an additional neuropathological process.

Lastly, anaemia may be a marker of the accelerated senescence associated with HIV. Since this accelerated cellular aging process has also been identified as a risk association for HAND, it is possible that anaemia and HAND can co-occur on the basis of a shared aetiological mechanism. (15)

The association between anaemia and HAND has not been explored in a South African study population in the era of ART. We consider this as an important research question because if anaemia is indeed a risk factor for HAND, even in the setting of established ART, then it may represent a potentially modifiable risk that can be prevented or treated. This it would have clear implications for the clinical management of HAND in South Africa.

This study aimed to investigate the potential association between anaemia and HAND in a HIV infected South African study population where participants were clinically stable and on established ART.

Methods

Study design

A cross-sectional secondary data analysis was performed to assess the association between serum haemoglobin level and neurocognitive function. One hundred and twenty-nine participants who had both neurocognitive test (global deficit score) and full blood count results performed as part of a randomised placebo controlled trial were included. (35)

Criteria for eligibility

For consent purposes, participants had to be older than 18 years and cognitively well enough to provide informed consent. All participants were receiving ART and they were otherwise clinically well with systemic viral suppression (HIV PCR <400 copies/mL). Participants were excluded if neuropsychiatric disorders, serious psychiatric symptoms or imaging structural abnormalities were present.

Study procedure

This has been previously published. (35) Phlebotomy for full blood counts was done at study entry. All blood samples were collected in appropriate EDTA tubes. An accredited South African pathology laboratory service provider was used. The automated processing of haematological samples was done by Advia machines. All results were signed out by qualified haematological pathologists. A stringent definition of anaemia, as reflected by a serum haemoglobin level of < 10 g/dL, was used. This definition was in keeping with the definition used by most South African studies investigating anaemia in HIV positive populations and was also in keeping with the WHO toxicity grading systems definition of anaemia. (36) The Global Deficit Score (GDS) has been validated for use in HIV and summarizes the neuropsychological test results of selected cognitive domains and adjusts for age, education, gender and ethnicity (43). Neurocognitive impairment was defined as a GDS score of greater than 0.5.

Statistical Analysis

An initial descriptive analysis was performed. Univariate analysis was then used to assess the association between GDS and variables of interest. T-tests and Chi-square tests were used as applicable. An attempt was made to log transform all nonparametric data in order to use parametric statistical tools. Where such transformations were not possible nonparametric statistical tools were used- namely Mann-Whitney and Fisher Exact tests. Statistical significance was set at the .05 level. Following the univariate analyses a regression analyses was performed, using GDS as the dependent variable. Thereafter the data was binarized on the basis of GDS using 0.05 as cutoff. Descriptive and cross-sectional analysis was performed by J Vermaak using STATA/IC 11. A final data analysis including the regression analyses was supervised by a UCT based statistician, Michelle Henry.

Results

Study population characteristics

A total of 129 study participants were included in the analysis. The majority were female (87%) and the mean age was 37 years (SD=7.78). (Table 4.1) The mean number of years of education was 9.44 years (SD=1.77). Participants were all established on ART and CD4 counts were generally well-preserved with a median of 495 cells/ μ L (IQR=315-629).

Haemoglobin and the distribution of haemoglobin

Overall the median haemoglobin was 12.2g/dL. (Table 4.1) Haemoglobin was not normally distributed and there was a narrow interquartile range of 11.6 to 13.0g/dL - mostly low normal. (Figure 4.1) Anaemia was present in 8.5% of the study population. The mean values for Mean corpuscular volume, 97.56 fL and Mean corpuscular haemoglobin, 31.23 pg were both within normal range.

Normal GDS versus impaired GDS

When assessing group differences between patients who had a normal GDS vs. those with GDS-defined neurocognitive impairment only age ($p=.003$) and years of education ($p=.002$) showed statistical significance on univariate analysis. (Table 4.2) Haemoglobin, red cell indices and Vitamin B 12 did not show association with GDS. (Figure 4.2)

A stepwise hierarchical regression found that only education significantly predicted GDS score ($R = .423$, $R^2 = .179$, $F [1,116] = 25.29$, $p < .001$) and explained 18% of the variance.

Participants with a higher education level had lower GDS scores, indicating better cognitive functioning. (Table 4.3)

Table 4.1 Study population characteristics (n = 129)

Mean age (SD)	37 (7.78)
Gender:	
Female (%)	112 (87)
Male (%)	17 (13)
Years of education (SD)	9.44 (1.77)
CD4 count, median (IQR)	495 (315-629)
Haemoglobin, median (IQR)	12.2 (11.6-13.00)
Anaemia (%)	11 (8.53)
Mean corpuscular volume, mean (range)	97.56 (62-125)
Mean corpuscular haemoglobin, mean (range)	31.23 (16-39)
Red Cell Count, mean (range)	3.93 (2.63-4.96)
Platelet count, mean (range)	281.11 (140-549)
Thyroid stimulating hormone, mean (range)	2.20 (0.27-18.17)
Vit B12, mean (range)	256.91 (89-523)
Global deficit score, mean (range)	1.04 (0-3.53)
GDS impaired (%)	96 (75)

SD = standard deviation, IQR = inter quartile range

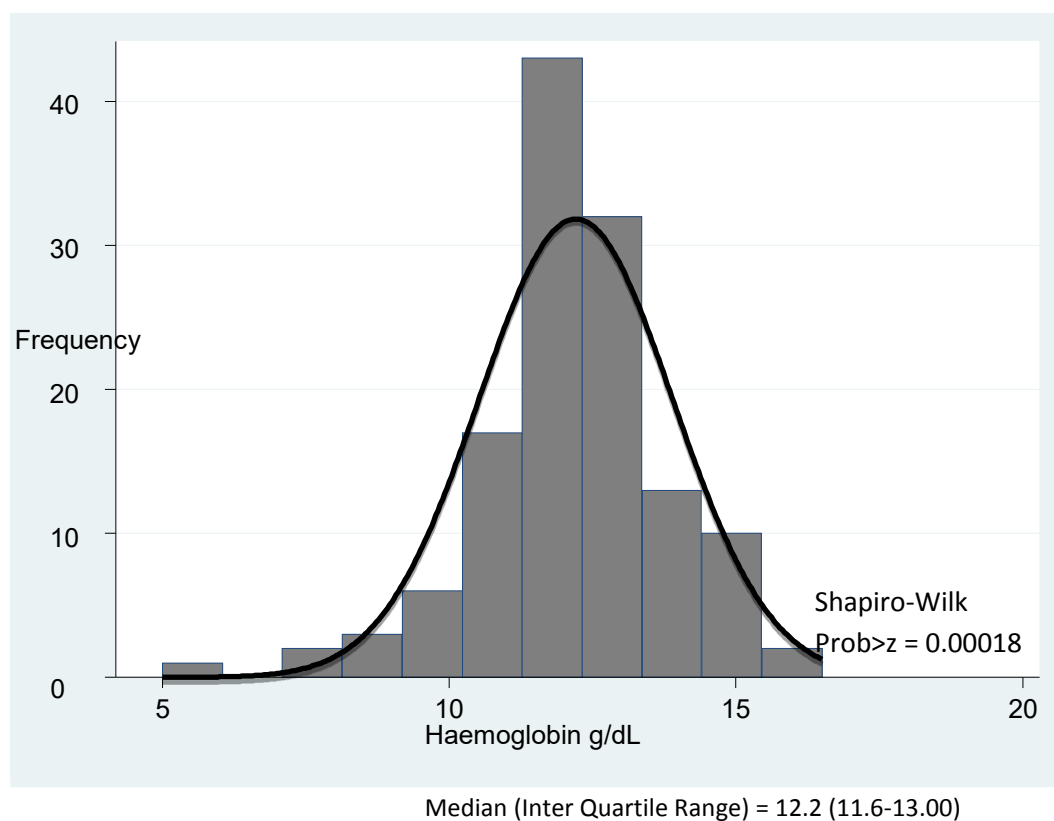


Figure 4.1 Distribution of Haemoglobin

Table 4.2 Comparison between participants with normal GDS vs. those with impaired GDS

	Normal GDS (<0.5) (N=32)	Impaired GDS (≥ 0.5) (N=97)	<i>p</i>
Age, mean (range)	34 (24-53)	39 (23-60)	.003
Gender:			
Female (%)	28 (88)	84 (87)	*
Male (%)	4 (13)	13 (13)	
Years of education (SD)	10.35 (1.80)	9.15 (1.66)	.002
CD4 count, median (IQR)	470 (318-626)	485 (299-633)	.94
Haemoglobin, median (IQR)	12.5 (11.6-13.3)	12.1 (11.6-12.9)	.31
Anaemia (%)	2 (6)	9 (9)	**
Mean corpuscular volume, mean (range)	97.38 (79-124)	97.62 (62-125)	.60
Mean corpuscular haemoglobin, mean (range)	31.56 (25-39)	31.12 (16-39)	.74
Red Cell Count, mean (range)	3.97 (3.12-4.96)	3.91 (2.63-4.93)	.53
Platelet count, mean (range)	273.81 (166-410)	283.52 (140-549)	.50
Thyroid stimulating hormone, mean (range)	2.71 (0.74-18.17)	2.04 (0.27-7.78)	.81
Vit B12, mean (range)	250.72 (119-466)	259 (89-523)	.93

Continuous variables shown as median (inter quartile range) * No significance difference for male and female distribution ($M = 0.89 \pm 0.54$ vs $M = 1.04 \pm 0.67$; $t(126) = -0.85$, $p = .397$)

** No significance between participants with or without GDS defined neurocognitive impairment (Odds Ratio[95% Confidence Interval] = 0.65[0.13-3.19])

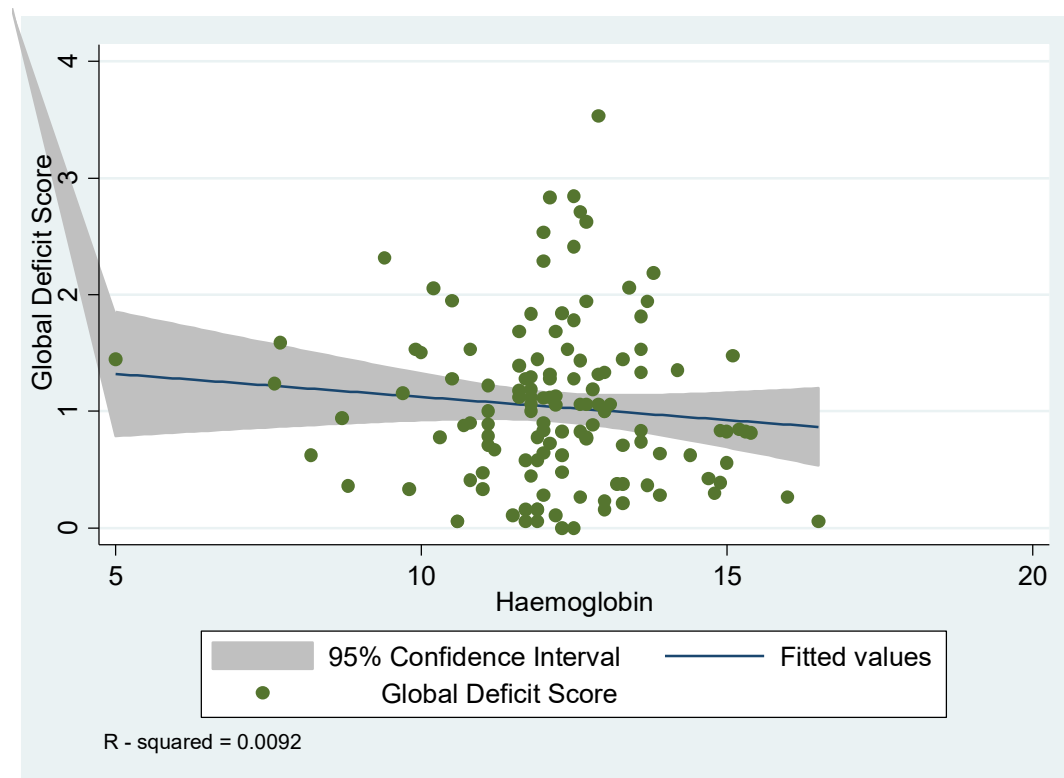


Figure 4.2 Scatterplot of Haemoglobin and GDS

Table 4.3 Correlation matrix between GDS and variables of interest

	GDS	Education	Age	CD4	RCC	Hb	MCV	MCH	Platelets	TSH	B12
GDS	1.000	-.423**	.264**	-.013	-.081	-.131	.037	-.066	.135	.094	-.090
Years of Education		1.000	-.308**	.221*	.047	.030	-.092	.010	-.113	-.172*	.128
Age			1.000	-.104	-.085	.001	.105	.089	.009	.086	-.066
CD4 count				1.000	.012	.031	.021	.071	.048	.001	.072
RCC					1.000	.669**	-.532**	-.349**	-.004	.027	.095
Hb						1.000	.202*	.446**	-.147	.036	-.059
MCV							1.000	.902**	-.111	.071	-.148
MCH								1.000	-.169*	.031	-.139
Platelets									1.000	-.048	.134
TSH										1.000	.002
B12											1.000

Note. * $p < .05$. ** $p < .001$.

Discussion

This cross-sectional study is the first African study to investigate the possible association between low serum haemoglobin (Hb) and HAND in an ART experienced and virologically suppressed population. Anaemia was present in 8.5% of our participants and serum haemoglobin level was not associated with GDS-defined neurocognitive impairment. Fewer years of education was the only independent risk association for GDS-defined neurocognitive impairment.

These findings are important because anaemia was a common finding in the pre-ART era with reported prevalence rates as high as 90%. Additionally, low haemoglobin represented a potential risk factor for neurocognitive impairment. Our study shows that amongst individuals who are established on ART, anaemia is now less common than in the pre-ART era and importantly, that low-normal serum Hb levels do not present a risk for GDS-defined neurocognitive impairment. Our results are particularly relevant as our South Africa study population is representative of the larger Sub-Saharan population of PLHIV, the global epicenter of HIV.

The 8.5% prevalence of anaemia that we identified was in keeping with the previously reported prevalence rates of 5.4% and 7.2% reported for two North American HIV positive female cohorts receiving ART. (13, 14) The fact that the frequencies of anaemia is similar in African and North American cohorts receiving ART suggests that good viral control is probably the most

important protective factor against HIV associated anaemia. This is in keeping with the known pathophysiology of HIV associated anaemia, where immune dysregulation is the main contributor to reduced Hb levels. In addition to this, our finding also suggests that the other common aetiologies for anaemia such as iron deficiency, Vit B12 deficiency and opportunistic infections are unlikely to be major contributors to anaemia in the context of established ART.

Our finding that low-normal serum Hb was not associated with GDS suggests that aggressive treatment of asymptomatic low-normal Hb levels is not indicated in otherwise well PLHIV. It further suggests that the potential physiological factors associated with low Hb, such as lower levels of erythropoietin, do not have a clinical effect on neurocognitive performance provided that Hb is in the low-normal range.

As noted during the pre-ART era, select studies showed association between anaemia and HAND. Our ART established study population showed significant differences to these studies with regard to both population and methodology. Firstly, because all our participants were established on ART, our participants had a much higher mean CD4 count than the participants in the pre-ART era. This is relevant because advanced immune compromise is a well recognised risk factor for both HAND and low Hb. (37, 15) Therefore, in the pre-ART studies the association between anaemia and HAND might have been due to confounding. Furthermore, we explored the association between Hb and GDS whereas most of the pre-ART studies examined the association between anaemia and severe neurocognitive impairment (HAD). It is also notable that our participants were predominantly women compared to the pre-ART study populations who were mostly male. This is of potential relevance because of the well-established differences in the normal Hb distribution of men and women, with women generally having a lower Hb. This difference in Hb distribution has led some to suggest that women, when compared to men, are physiologically superior with regards to functioning at low or low-normal Hb levels. (38) It is therefore possible that our participants were potentially physiologically less affected by their low-normal Hb levels because they were female. Lastly our study was done in Sub-Saharan Africa and therefore participants were most likely infected by HIV-1 clade C. Contrastingly, most of the pre-ART studies were done in North America where clade B predominates. Different clades of the HIV virus exhibit different degrees of neurotoxicity with clade C (Sub-Saharan Africa and Southern Asia) being less neurotoxic than clade B. (39) More recent in vitro research has shown that there are differences in the HIV-1 Tat protein and specifically the dicysteine motif in Tat that is responsible for clade related differences in neurotoxicity. (40) To the best of our knowledge the clade related effects on Hb and the risk for anaemia have not been explored.

In our study, fewer years of education was the only factor which was independently associated with poorer GDS scores. This finding was not unexpected as education level has a known effect on neuropsychological test performance. (41) Higher levels of education may also increase neurocognitive reserve and as such, convey a degree of protection against neurocognitive impairment. (42)

Limitations to our study include: patient selection and limited data for the aetiology of low Hb levels. We used data from an established research project where individuals who thought they may have some degree of neurocognitive impairment were invited to formal GDS screening. (35) This may have resulted in a greater proportion of our participants having abnormal GDS scores. However, because we primarily assessed association with GDS as a continuous variable, we believe that our exploratory analysis was sufficient to test our study hypothesis. Secondly, although our study examined Hb level, red cell indices and Vitamin B12, the Li in HAND study did not measure iron or folate levels. This limited the conclusions that we could draw with regards to the aetiology of anaemia in our study.

Our cross-sectional research focused on the association between Hb and neurocognitive function in the context of established ART. We suggest that future research explores the effects of ART initiation on Hb levels and the longitudinal association between Hb level and neurocognitive outcomes. It would be of particular interest to investigate whether symptomatically low levels of Hb are associated with the later development of neurocognitive symptoms.

Conclusion

Our study shows that in South Africans who are well established on ART, anaemia is now less common than in the pre-ART era and importantly that low-normal serum Hb levels do not present a risk for GDS-defined neurocognitive impairment. These findings are relevant as they show that aggressive management of low-normal Hb levels is not necessary if individuals are otherwise clinically well and virally suppressed.

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